Claims

1. A method for confirming hydrogen/deuterium exchange in the structure of a biological sample, comprising the following steps:

(1) selecting a known natural protein as matrix, and formulating it with PBS to form a matrix solution having an appropriate concentration;

(2) subjecting a deuterated biological sample to be confirmed and a corresponding natural biological sample respectively to the following manipulations: diluting the sample obtained after purification with PBS to form a diluted sample having a desired concentration, mixing homogeneously the diluted sample with a suitable amount of the matrix solution followed by lyophilization to form a lyophilized mixed sample, sufficiently oxidizing the lyophilized mixed sample with oxidants through burning in order to oxidize the hydrogen in the mixed sample to water, reacting the resulting water after separation with zinc to generate hydrogen gas, and determining the $^{2}H/^{1}H$ ratio of the generated hydrogen gas with a gas isotope mass spectrometer;

(3) comparing the determined $^{2}H/^{1}H$ ratio of the generated hydrogen gas of the deuterated biological sample to be confirmed with that of the corresponding natural biological sample, wherein if the $^{2}H/^{1}H$ ratio of the generated hydrogen gas of the deuterated biological sample to be confirmed is significantly higher than that of the corresponding natural biological sample, it is confirmed that hydrogen/deuterium exchange has occurred in the structure of the deuterated biological sample to be confirmed.

2. The method for confirming hydrogen/deuterium exchange in the structure of a biological sample according to claim 1, characterized in that said known natural protein is bovine serum albumin (BSA), egg albumin or lysozyme.

3. The method for confirming hydrogen/deuterium exchange in the structure of a biological sample according to claim 1, characterized in that said biological sample is a microorganism sample or a biomacromolecule sample.

4. The method for confirming hydrogen/deuterium exchange in the structure of a biological sample according to claim 3, characterized in that said biomacromolecule is a ribonucleic acid (RNA), a protein or a polypeptide.

5. Use of the method for confirming hydrogen/deuterium exchange in the structure of a biological sample according to claim 1 in confirming the close correlation between the stability and heat resistance of microorganisms or biomacromolecules and the deuterium/hydrogen ratio thereof.

6. A method for determining the percent content of deuterium in the structure of a biological sample, comprising the following steps:

(1) selecting a known natural protein as matrix, and formulating it with PBS to form a matrix solution having an appropriate concentration;

(2) diluting a biological sample obtained after purification with PBS to various concentrations, subjecting multiple sample solutions that have different concentrations but the same volume respectively to the following manipulations: mixing homogeneously the sample solution with a suitable amount of the matrix solution followed by lyophilization to form a lyophilized mixed sample, sufficiently oxidizing the lyophilized mixed sample with oxidants through burning in order to oxidize the hydrogen in the mixed sample to water, reacting the resulting water after separation with zinc to generate hydrogen gas, and determining the $^2H/^1H$ ratio of the generated hydrogen gas with a gas isotope mass spectrometer; and

(3) according to a formula, calculating the percent content of deuterium in the biological sample by utilizing the above $^2H/^1H$ ratios of hydrogen gas determined for the multiple samples.

7. The method for determining the percent content of deuterium in the structure of a biological sample according to claim 6, characterized in that said step (3) is carried out as follows:

(a) plotting the determined $^2H/^1H$ ratios of the multiple samples (expressed with $\delta D_{SA\text{-}SMOW}$) against the amount of biological sample/amount of matrix protein (expressed with m_{bio}/m_{MAT}), and thus obtaining a straight line having a good linear correlation, $\delta D_{SA-SMOW} = k \cdot \frac{m_{bio}}{m_{MAT}} + \delta D_{MAT-SMOW}$, and educing the slope of the straight line, $k = \frac{500 \cdot C_{bio}^{D}}{R_{SMOW} \cdot C_{MAT}^{H}}$;

(b) calculating the theoretical percent content of ^1H in the matrix protein, C_{MAT}^{H}, according to the amino acid composition of the

matrix protein;

(c) according to the slope k, calculating the percent content of deuterium in the biological sample as $C^{D}_{bio}=k\times R_{SMOW}\times C^{H}_{MAT}\times\frac{1}{500}$, wherein R_{SMOW} represents the $^{2}H/^{1}H$ ratio of SMOW and its value is 155.76×10^{-6}.

8. The method for determining the percent content of deuterium in the structure of a biological sample according to claim 6, characterized in that said known natural protein is bovine serum albumin (BSA), egg albumin or lysozyme.

9. The method for determining the percent content of deuterium in the structure of a biological sample according to claim 6, characterized in that said biological sample is a microorganism sample or a biomacromolecule sample.

10. The method for determining the percent content of deuterium in the structure of a biological sample according to claim 9, characterized in that said biomacromolecule is a ribonucleic acid (RNA), a protein or a polypeptide.

11. Use of the method for determining the percent content of deuterium in the structure of a biological sample according to claim 6 or 7 in ascertaining the deuterium content of a biological sample having the optimal heat stability or ascertaining the optimal deuteration conditions of a biological sample.

12. Use of the method for determining the percent content of deuterium in the structure of a biological sample according to claim 6 or 7 in confirming the close correlation between the stability and heat resistance of a microorganism or a biomacromolecule and the deuterium content thereof.